

## VI. CLAIMS

We claim:

- 5 1. A method of separating sperm cells, comprising:
  - a. obtaining semen from a male of a species of mammal which contains a plurality of sperm cells;
  - b. incubating said semen at a temperature above which sperm cell membrane lipids transition from a liquid phase to gel phase;
  - 10 c. determining a sperm cell characteristic of a plurality of said sperm cells;
  - d. separating said sperm cells based upon said sperm cell characteristic; and
  - e. collecting separated sperm cells.
2. A method of separating sperm cells as described in claim 1, wherein said  
15 temperature above which sperm cell membrane lipids transition from a liquid phase to gel phase comprises the step of incubating said semen at a temperature above which sperm cell membrane lipids transition to a gel phase.
3. A method of separating sperm cells as described in claim 1, wherein said  
20 temperature above which sperm cell membrane lipids transition from a liquid phase to gel phase comprises the step of incubating said semen at a temperature which maintains said sperm cell membrane lipids in said liquid phase.
4. A method of separating sperm cells as described in claim 1, wherein said step of  
25 incubating said semen at a temperature above which sperm cell membrane lipids transition from a liquid phase to gel phase temperature comprises incubating said semen at a temperature between about 5° C and about 25° C.
5. A method of separating sperm cells as described in claim 1, wherein said  
30 temperature is selected from the group consisting of about 5 ° C, about 6° C, about 7° C, about 8° C, about 9° C, about 10° C, about 11° C, about 12° C, about 13° C, about 14° C, about 15° C, about 16° C, about 17° C, about 18° C, about 19° C, about 20° C, about 21° C, about 22° C, about 23° C, about 24° C, and about 25° C.

6. A method of separating sperm cells as described in claim 1, wherein said species of mammal is selected from the group consisting of a bovine species of mammal, an equine species of mammal, an ovine species of mammal, a swine species of mammal, a canine species of mammal, a feline species of mammal, a deer species of mammal, an elk species of mammal, and a marine species of mammal.
7. A method of separating sperm cells as described in claim 1, wherein said species of mammal comprises a bovine species and wherein said step of incubating said semen at a temperature above which sperm cell membrane lipids transition from a liquid phase to gel phase temperature comprises incubating said semen at a temperature between about 17° C and about 19° C.
8. A method of separating sperm cells as described in claim 1, wherein said species of mammal comprises a bovine species and wherein said step of incubating said semen at a temperature above which sperm cell membrane lipids transition from a liquid phase to gel phase temperature comprises incubating said semen at a temperature of about 17° C.
9. A method of separating sperm cells as described in claim 1, wherein said species of mammal comprises an equine species and wherein said step of incubating said semen at a temperature above which sperm cell membrane lipids transition from a liquid phase to gel phase temperature comprises incubating said semen at a temperature of about 15° C.
10. A method of separating sperm cells as described in claim 1, wherein said step of incubating said semen at a temperature above which sperm cell membrane lipids transition from liquid phase to gel phase comprises incubating said semen at said temperature above which sperm cell membrane lipids transition from liquid phase to gel phase between about one hour to about 18 hours.
11. A method of separating sperm cells as described in claim 1, further comprising the step of transporting said semen from a first location to a second location during said step of incubating said semen at a temperature above which sperm cell membrane lipids transition from a liquid phase to gel phase.
12. A method of separating sperm cells as described in claim 1, further comprising the step of adding an antibacterial to said semen prior to said step of incubating said semen at

a temperature above which sperm cell membrane lipids transition from a liquid phase to gel phase.

13. A method of separating sperm cells as described in claim 1, wherein said step of  
5 determining a sperm cell characteristic of a plurality of sperm cells within said semen  
comprises determining a sex characteristic of said sperm cells.

14. A method of separating sperm cells as described in claim 1, wherein said step of  
separating said sperm cells based upon said sperm cell characteristic comprises separating  
10 said sperm cells based upon said sex characteristic.

15. A method of separating sperm cells as described in claim 1, further comprising the  
step of extending semen with an extender selected from the group consisting of KMT, and  
INRA96.  
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16. A method of separating sperm cells as described in claim 1, further comprising the  
step of concentrating said sperm cells by removing a portion of seminal plasma.

17. A method of separating sperm cells as described in claim 1, further comprising the  
20 step of staining said sperm cells.

18. A method of separating sperm cells as described in claim 17, wherein said step of  
staining said sperm cells comprises staining DNA contained within said sperm cells.

25 19. A method of separating sperm cells as described in claim 18, wherein said step of  
staining DNA contained within said sperm cells comprises staining said DNA within said  
sperm cells with Hoechst 33342 stain.

20. A method of separating sperm cells as described in claim 19, wherein said step of  
30 staining said DNA within said sperm cells with Hoechst 33342 stain comprises incubating  
said sperm cells with Hoechst 33342 for a period of between about 30 minutes and about  
1 hour.

21. A method of separating sperm cells as described in claim 1, wherein said step of  
35 separating said sperm cells based upon said sperm cell characteristic comprises separating

said sperm cells using an instrument selected from the group consisting of a flow cytometer, and a cell sorter.

22. An artificial insemination sample, comprising:

- 5 a. an artificial insemination device;
- b. a sperm cell extender; and
- 10 c. semen obtained from a male of a species of mammal containing a plurality of sperm cells incubated at a temperature above which sperm cell membrane lipids transition from a liquid phase to a gel phase for a period of between one hour and 18 hours, and wherein said plurality of sperm cells are separated on the basis of a sex characteristic, and wherein a portion of said separated sperm cells are established in said sperm cell extender, and wherein said portion of said separated sperm cells established in said sperm cell extender are contained within said artificial insemination device.

15 23. An artificial insemination sample as described in claim 22, wherein said sperm cell extender comprises an egg yolk extender.

24. An artificial insemination sample as described in claim 22, wherein said sperm cell  
20 extender comprises an egg yolk-TRIS extender.

25. An artificial insemination sample as described in claim 22, wherein said sperm cell extender comprises substantially equal volumes of said egg yolk-TRIS extender and egg yolk-TRIS extender/12% glycerol.

25 26. An artificial insemination sample as described in claims 23, 24, or 25, wherein said sperm cell extender has a volume of between about 0.1 millilitre and about 2.0 millilitre.

27. An artificial insemination sample as described in claim 22, wherein said artificial  
30 insemination device comprises an artificial insemination straw.

28. An artificial insemination sample as described in claim 22, wherein said portion of said separated sperm cells established in said sperm cell extender comprise between about one hundred thousand separated sperm cells and about 25 million separated sperm cells.

29. An artificial insemination sample as described in claim 28, wherein said portion of said separated sperm cells established in said sperm cell extender contained within said artificial insemination device is frozen.
- 5 30. An artificial insemination sample as described in claim 29, wherein said portion of said separated sperm cells frozen in said sperm cell extender contained within said artificial insemination device are thawed.
31. A method of producing an artificial insemination sample comprising the steps of:
- 10 a. obtaining semen from a male of a species of mammal which contains a plurality of sperm cells;
- b. incubating said semen at a temperature above which sperm cell membrane lipids transition from a liquid phase to a gel phase;
- c. determining a sex characteristic of said plurality of sperm cells;
- 15 d. separating said plurality of sperm cells based upon said sex characteristic; and
- e. establishing an artificial insemination sample containing separated sperm cells capable of fertilizing at least one egg within a female of said species of said mammal.
- 20 32. A method of fertilizing a mammal comprising the steps of:
- a. obtaining semen from a male of a species of mammal which contains a plurality of sperm cells;
- b. incubating said semen at a temperature above which sperm cell membrane
- 25 lipids transition to a gel phase;
- c. determining a sex characteristic of said plurality of sperm cells;
- d. separating said plurality of sperm cells based upon said sex characteristic;
- e. establishing an artificial insemination sample containing separated sperm cells capable of fertilizing at least one egg within a female of said species
- 30 of said mammal;
- f. inserting a portion of said insemination sample into a female of said species of said mammal; and
- g. fertilizing at least one egg within said female of said species of said mammal.
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33. A method of producing an offspring mammal comprising the steps of:
- a. obtaining semen from a male of a species of mammal which contains a plurality of sperm cells;
  - b. incubating said semen at a temperature above which sperm cell membrane lipids transition to a gel phase;
  - c. determining a sex characteristic of said plurality of sperm cells;
  - d. separating said plurality of sperm cells based upon said sex characteristic;
  - e. establishing an artificial insemination sample containing separated sperm cells capable of fertilizing at least one egg within a female of said species of said mammal;
  - f. inserting a portion of said insemination sample into a female of said species of said mammal;
  - g. fertilizing at least one egg within said female of said species of said mammal; and
  - h. producing an offspring mammal.